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Biological evaluation of medical devices —

Part 13: Identification and quantification of degradation products from polymeric medical devices

Évaluation biologique des dispositifs médicaux —

Partie 13: Identification et quantification de produits de dégradation de dispositifs médicaux à base de polymères

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 10993-13 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This second edition cancels and replaces the first edition (ISO 10993-13:1998), which has been technically revised.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1: Evaluation and testing within a risk management process*

— *Part 2: Animal welfare requirements*

— *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4: Selection of tests for interactions with blood*

— *Part 5: Tests for* in vitro *cytotoxicity*

— *Part 6: Tests for local effects after implantation*

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 9: Framework for identification and quantification of potential degradation products*

— *Part 10: Tests for irritation and skin sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymeric medical devices*

— *Part 14: Identification and quantification of degradation products from ceramics*

— *Part 15: Identification and quantification of degradation products from metals and alloys*

— *Part 16: Toxicokinetic study design for degradation products and leachables*

— *Part 17: Establishment of allowable limits for leachable substances*

— *Part 18: Chemical characterization of materials*

— *Part 19: Physico-chemical, morphological and topographical characterization of materials* [Technical specification]

— *Part 20: Principles and methods for immunotoxicology testing of medical devices* [Technical specification]

Introduction

Degradation products covered by this part of ISO 10993 are formed primarily by chemical bond scission due to hydrolytic and/or oxidative processes in an aqueous environment such as the human body. It is recognised that additional biological factors, such as enzymes, other proteins and cellular activity, can alter the rate and nature of degradation.

It should be kept in mind that a polymeric device can contain residuals and leachables such as monomers, oligomers, solvents, catalysts, additives, fillers and processing aids. These components which, if present, can interfere with the identification and quantification of the degradation products need to be considered and accounted for. It should be recognised that residual monomers can generate the same degradation products as the polymer itself. If the reader is solely interested in using the results from a degradation test as input to further biological evaluation tests, the reader might not be interested in distinguishing between a leachable and a degradation product. If this is the case, then the care taken to separate the leachable from the degradation product may not be needed.

Because of the generalized nature of this part of ISO 10993, product standards, when available, that address degradation product formation under more relevant conditions of use, may be considered as an alternative. This part of ISO 10993 is suitable for screening new polymeric materials and/or modified polymeric materials with unknown degradation behaviour in body contact. This part of ISO 10993 does not reproduce degradation *in vivo*. The user of this part of ISO 10993 can consider running additional degradation tests addressing *in vivo* degradation issues.

Long-term implants might not degrade within the time frame of the tests shown in this part of ISO 10993. The intention of this part of ISO 10993 is to help determine the biological hazards from potential degradation products from polymer components of medical devices. As noted above, those products might come from a variety of degradation mechanisms. This part of ISO 10993 is not intended to be a complete analysis of the degradation of the medical device and the impact on its performance. The interested user is referred to the relevant product standards.

The identified and quantified degradation products form the basis for biological evaluation in accordance with ISO 10993-1, for risk assessment in accordance with ISO 10993-17 and, if appropriate, for toxicokinetic studies in accordance with ISO 10993-16.

Biological evaluation of medical devices —

Part 13:
Identification and quantification of degradation products from polymeric medical devices

1 Scope

This part of ISO 10993 provides general requirements for the design of tests in a simulated environment for identifying and quantifying degradation products from finished polymeric medical devices ready for clinical use.

This part of ISO 10993 describes two test methods to generate degradation products, an accelerated degradation test as a screening method and a real-time degradation test in a simulated environment. For materials that are intended to polymerize *in situ*, the set or cured polymer is used for testing. The data generated are used in the biological evaluation of the polymer. This part of ISO 10993 considers only non-resorbable polymers. Similar but appropriately modified procedures may be applicable for resorbable polymers.

This part of ISO 10993 considers only those degradation products generated by a chemical alteration of the finished polymeric device. It is not applicable to degradation of the device induced during its intended use by mechanical stress, wear or electromagnetic radiation or biological factors such as enzymes, other proteins and cellular activity.

NOTE An informative text discussing environmental stress cracking (ESC) of polymers is included as a potential aid to the design of degradation studies (see Annex B).

The biological activity of the debris and soluble degradation products is not addressed in this part of ISO 10993, but should be evaluated according to the principles of ISO 10993-1, ISO 10993-16 and ISO 10993-17.

Because of the wide range of polymeric materials used in medical devices, no specific analytical techniques are identified or given preference. No specific requirements for acceptable levels of degradation products are provided in this part of ISO 10993.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

ISO 10993-9, *Biological evaluation of medical devices — Part 9: Framework for identification and quantification of potential degradation products*

ISO 10993-12, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO 10993-17, *Biological evaluation of medical devices — Part 17: Establishment of allowable limits for leachable substances*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
residual monomer
unreacted chemical compound(s) used to build the polymeric chains, which is still present in the final polymeric material

3.2
degradation product
chemical compound derived from the breakdown of the polymeric material, including any compound produced by consecutive chemical reactions

3.3
polymeric material
materials consisting of long-chain and/or crosslinked molecules composed of units called monomers

3.4
hydrolytic degradation
scission of chemical bonds in a polymer by the attack of water

NOTE The water can have a neutral, acidic or alkaline pH value and can contain additional chemical compounds or ions.

3.5
oxidative degradation
scission of chemical bonds in a polymer by the attack of one or more oxidizing agents

3.6
debris
particulate material produced by the degradation of a polymeric material

4 Degradation test methods

4.1 General procedures

4.1.1 Test design

In accordance with ISO 10993-9, degradation tests shall be used to generate, identify and/or quantify degradation products. If degradation is observed in an accelerated test, identification and quantification of the degradation products can provide sufficient information for risk analysis. If identification and quantification of degradation products from the accelerated test do not provide sufficient information for the risk analysis, real-time testing shall be performed. The sequence of steps that shall be followed is described in detail in this part of ISO 10993.

NOTE The accelerated degradation test can be used as a screening test. If no degradation is observed in the accelerated test, no real-time degradation test should be necessary.

4.1.2 Sample preparation

When not specifically addressed by the selected method(s), the general aspects of sample preparation shall be in accordance with ISO 10993-12.

4.1.3 Initial material characterization

The analytical methods used for the initial material characterization shall be appropriate for the polymeric material under investigation. The analytical techniques used shall be reported and justified.

Annex A presents a list of analytical methods and their application range for the characterization of polymeric materials.

4.1.4 Test solutions and apparatus

4.1.4.1 Test solutions

4.1.4.1.1 General

All test solution(s) used shall be described and justified in the test report.

The test solution shall be selected to be as similar as possible to the intended environment in which the polymeric medical device is going to be used.

If the service environment cannot be simulated, test solutions given in 4.1.4.1.2 and 4.1.4.1.3 can be used as a first screening for degradation. These test solutions can be more challenging or less challenging to the polymeric material with respect to the intended degradation mechanisms than the *in vivo* environment.

Other test solutions for a specific polymer or a specific service environment may be chosen.

NOTE If a biological assay of the debris or the degradation solution is to be made, then the use of antibacterial or antifungal additives will interfere with these assays and it might be necessary to maintain a sterile environment for the duration of the real-time degradation test.

4.1.4.1.2 Test solutions for hydrolytic degradation

For hydrolytic degradation, the following solutions are suggested:

a) water for analytical laboratory use, grade 2, in accordance with ISO 3696;

b) buffer.

NOTE See ISO 13781 for examples of buffers used in hydrolytic degradation studies.

4.1.4.1.3 Test solutions for oxidative degradation

For oxidative degradation, the following solutions are suggested:

a) water and hydrogen peroxide, e.g. 3 % hydrogen peroxide solution, Pharmacopoeia grade;

b) Fenton's reagent [mixture of dilute hydrogen peroxide solution and iron(II) salts, e.g. 100 µmol Fe^{2+} and 1 mmol H_2O_2].

These oxidative solutions might not be stable at elevated temperatures or for a prolonged time. Therefore, the oxidative capacity shall be maintained in an appropriate range.

This stability range shall be specified, justified and reported.

4.1.4.2 Container

Depending on the test solution, chemical grade glassware, polytetrafluoroethylene or polypropylene containers in an enclosed system shall be used. Controls shall be used in order to assess contaminants from the container. Evidence shall be provided that containers do not interfere with the analysis.

4.1.4.3 Balance

The balance used to determine mass loss shall be capable of weighing the initial sample mass with the precision required. For materials designed to be resorbed, a precision of 1 % is appropriate, for materials designed to resist degradation, a precision of at least 0,1 % shall be used. The precision of the balance for the final sample mass in the case of resorbable polymers shall be 0,1 %, and in the case of stable polymers 0,01 %, of the total sample mass.

The precision and standard deviation of the method used for determining mass loss shall be stated in the test report.

4.1.4.4 Drying apparatus

Any apparatus capable of drying the test samples to constant mass without contamination or loss of volatile degradation products shall be used.

The apparatus shall be described and defined in the test report.

4.1.4.5 Vacuum source

Any apparatus capable of producing a sufficient vacuum (< 0,5 kPa) in the drying apparatus is appropriate.

The apparatus shall be described and defined in the test report.

4.1.4.6 Separation apparatus

Any apparatus capable of separating the debris produced during the degradation study may be used. This can involve an inert filter, a temperature-controlled centrifuge or a combination thereof.

The apparatus shall be described and defined in the test report.

4.1.5 Number of test samples

At least three test samples shall be used for each test period. These should be the finished product itself or representative samples thereof. A separate container shall be used for each sample. One blank shall be used for each test period.

If a valid statistical analysis is required, more samples at each test period should be used, as appropriate.

4.1.6 Shape and size of test samples

The size and the shape of the specimen are critical for the generation of relevant amounts of degradation products. If a part of the finished device is used as the test sample, then surfaces which are normally not in contact with the biological environment should be avoided or minimized.

The size, shape and surface area of the sample should be chosen in such a way that equilibrium with the degradation solution and a constant mass for the determination of the mass balance can be reached in an acceptable time.

If the medical device consists of more than one material, combination effects should be taken into consideration. It is recommended that representative parts of the other materials of the device, not intended to be tested by this part of ISO 10993, should be added to the test solution.

NOTE 1 Under certain circumstances, it might be necessary to fabricate a test sample using the same processing, cleaning and sterilization methods as are used in the fabrication of the device.

NOTE 2 With resorbable polymers, equilibrium with the degradation solution might not be reached.

4.1.7 Mass/volume ratio

The ratio of the mass of the test sample to the volume of the test solution should be at least 1 g:10 ml. The samples shall be fully immersed in the test solution.

The choice of the ratio used shall be reported and justified in the test report.

The ratio 1 g:10 ml was chosen for practical reasons. When using this ratio, however, it should be considered that the release of degradation products can interfere with the progress of degradation itself and can influence the rate of the degradation and the equilibrium of the degradation reaction(s).

4.1.8 Sample pre-treatment

To set up the mass balance, the sample shall be dried to a constant mass. If the device contains volatile components, an appropriate drying method shall be selected.

In this case, the drying method and the conditions shall be stated and justified in the test report.

4.1.9 pH range

If the pH of the test solution is relevant, the pH shall be maintained in an appropriate range. The pH chosen shall be appropriate to the site of intended use (e.g. the acidic stomach). Changes in the pH induced by physiological phenomena, e.g. during an inflammatory response, shall be considered.

The pH shall be reported and justified in the test report.

NOTE Elevated temperatures can change the pH value.

It should be recognised that, if the pH value is not maintained in the appropriate range, the degradation products generated might or might not be the same as those that occur under biological conditions.

4.1.10 Determination of the mass balance

When the sample is removed from the test solution, the sample shall be rinsed with adequate quantities of analytical grade water. The rinse water and any debris loosened by the rinse water shall be added to the test solution. The sample and the debris eventually obtained from the separation apparatus shall be dried to a constant mass. Then the mass balance shall be determined.

NOTE A large quantity of rinsing water can preclude analysis of the fluid phase for leachables due to dilution.

4.1.11 Final material characterization

The material shall be characterized using the same methods as used in the initial material characterization (see 4.1.3).

4.2 Accelerated degradation test

4.2.1 Temperature

Choose a temperature greater than 37 °C but below the melting or softening range of the polymer. When appropriate, (70 ± 2) °C shall be used.

The temperature chosen shall be reported and justified in the test report.

NOTE 1 Higher temperatures can lead to side reactions and changes in the solubility of additives which might not occur at lower temperatures. Consideration of the thermal properties of the additives in the polymeric material is recommended.

NOTE 2 Differential scanning calorimetry can be used to obtain information about the melting range.

4.2.2 Test periods

For devices whose intended use is longer than 30 d, test periods of 2 d and 60 d shall be used. For devices whose intended use is less than 30 d, test periods of 2 d and 7 d shall be used. Additional test periods may be chosen depending on the polymer under investigation or the intended use of the device.

If the selected temperature is not 70 °C, the test period can require adjustment.

The test periods shall be reported and justified.

NOTE For devices made from resorbable polymers, this test period can last until the device has lost its integrity (as defined for the individual material).

4.3 Real-time degradation test in a simulated environment

4.3.1 Temperature

Carry out the test at (37 ± 1) °C.

4.3.2 Test period

For devices whose intended use is longer than 30 d, test periods of 1 month, 3 months, 6 months and 12 months shall be used. For devices whose intended use is less than 30 d, four alternative test periods shall be used, including 30 d. Additional test periods may be chosen depending on the polymer under investigation or the intended use of the device.

The test periods shall be reported and justified.

NOTE For devices made from resorbable polymers, this test period can last until the device has lost its integrity (as defined for the individual material).

5 Test procedures

5.1 General

The steps to be followed are described in 5.2, 5.3 and Figure 1.

NOTE For evaluation of crosslinked polymer systems, the decision for further action will be based on the mass balance calculation and a measurement of the density of crosslinking instead of molecular mass/molecular mass distribution determination.

5.2 Initial material characterization

The initial material characterization shall address the bulk polymer and the residuals and additives present in the final device. Because of the difficulties of retrospective analysis, this information is best obtained from the supplier or manufacturer of the material. It is important to fully characterize the purity of the polymer and the additives used in its formulation (see 4.1.3).

5.3 Accelerated degradation test

5.3.1 Measurement of initial mass

Dry the test sample to constant mass. Determine the mass of the test sample.

The drying method and the conditions shall be stated and justified in the test report.

5.3.2 Separation of sample, debris and solution

5.3.2.1 Separation by filtering

Dry a filter, under vacuum at room temperature, to constant mass. Determine the mass of the filter. Separate sample with possible debris from the degradation solution by means of the weighed filter. If necessary, vacuum or pressure filtering can be used. Wash the contents of the filter three times with analytical grade water.

5.3.2.2 Separation by centrifuging

Determine the mass of a dry and clean centrifuge tube. Transfer the degradation test sample solution into the centrifuge tube and close the tube prior to separation. Spin the tube in the centrifuge to obtain a firm debris pellet. Carefully decant the supernatant solution into a container. Resuspend the pellet in analytical grade water and spin again. Decant the supernatant solution again and add this solution to the container. Repeat this procedure two more times.

5.3.3 Analysis

5.3.3.1 Determination of mass balance

Dry the filter and its contents or the centrifuge tube and its contents, under vacuum at room temperature, to a constant mass. Determine the mass of the filter and its contents or the centrifuge tube and its contents. Determine the mass loss of the sample.

5.3.3.2 Final material characterization (sample and debris)

The molecular mass and the molecular mass distribution are determined by appropriate methods (see 4.1.11).

5.3.4 Evaluation

5.3.4.1 General

A flow chart for the test procedure to be followed is given in Figure 1.

5.3.4.2 Case 1 (No/No)

No change in mass balance and molecular mass/distribution.

No degradation has been observed. The test is terminated; no real-time degradation test is necessary.

NOTE Under some circumstances, it can be necessary to confirm this result by further investigations in line with ISO 10993-9.

5.3.4.3 Case 2 (No/Yes)

No change in mass balance, but molecular mass/distribution has changed. Check the bulk sample and debris for degradation products. Proceed with real-time degradation test, if necessary.

5.3.4.4 Case 3 (Yes/No)

Change in mass balance, but no change in molecular mass/distribution.

Polymer is not degraded, fluid phase contains leachables which shall be assessed according to other relevant parts of ISO 10993. Proceed with real-time degradation test, if necessary.

NOTE Leachables might not be generated at lower temperatures. Depending on the risk assessment of the leachables, real-time degradation tests in a simulated environment might be considered.

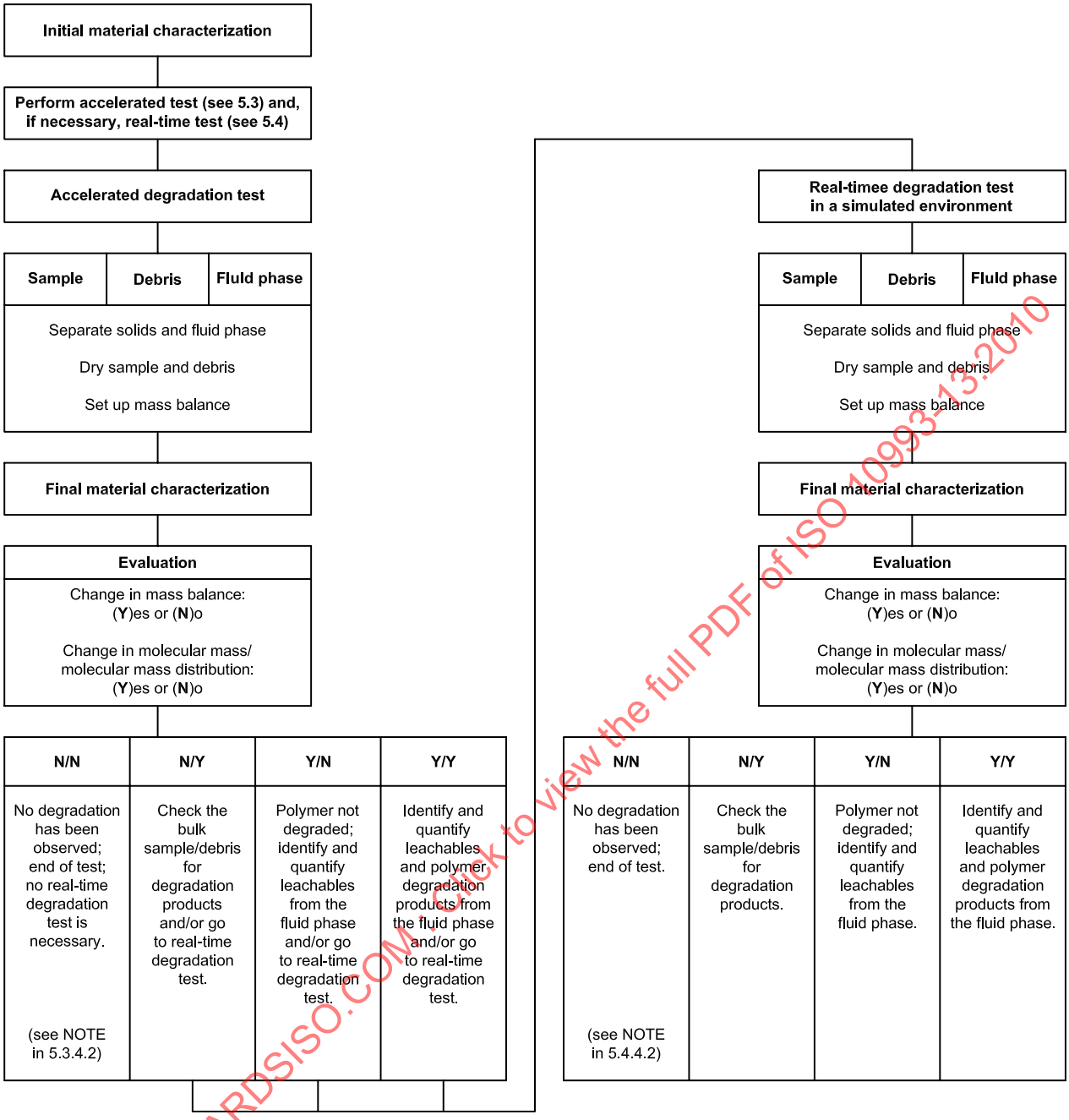

Figure 1 — Flow chart test procedures

5.3.4.5 Case 4 (Yes/Yes)

Change in mass balance and change in molecular mass/distribution.

Identify and quantify leachables and polymer degradation products from the fluid phase and check the bulk sample and debris for degradation products. Proceed with real-time degradation test, if necessary.

NOTE Degradation and leaching might not occur at lower temperatures. Depending on the risk assessment of the leachables and polymeric degradation products, real-time degradation tests in a simulated environment might be considered.

5.4 Real-time degradation test in a simulated environment

5.4.1 Measurement of initial mass

Dry the test sample to constant mass. Determine the mass of the test sample.

5.4.2 Separation of sample, debris and solution

5.4.2.1 Separation by filtering

Dry a filter, under vacuum at room temperature, to constant mass. Determine the mass of the filter. Separate sample with possible debris from the degradation solution by means of the weighed filter. If necessary, vacuum or pressure filtering can be used. Wash the contents of the filter three times with analytical grade water.

5.4.2.2 Separation by centrifuging

Determine the mass of a clean, dry centrifuge tube. Transfer the degradation test sample solution into the centrifuge tube and close the tube prior to separation. Spin the tube in the centrifuge to obtain a firm debris pellet. Carefully decant the supernatant solution into a container. Resuspend the pellet in analytical grade water and spin again. Decant the supernatant solution again and add this solution to the container. Repeat this procedure two more times.

5.4.3 Analysis

5.4.3.1 Determination of mass balance

Dry the filter and its contents or the centrifuge tube and its contents, under vacuum at room temperature, to a constant mass. Determine the mass of the filter and its contents or the centrifuge tube and its contents. Determine the mass loss of the sample.

5.4.3.2 Final material characterization (sample and debris)

The molecular mass and the molecular mass distribution are determined by appropriate methods (see 4.1.11).

5.4.4 Evaluation

5.4.4.1 General

A flow chart for the test procedure to be followed is given in Figure 1.

5.4.4.2 Case 1 (No/No)

No change in mass balance and molecular mass/distribution.

No degradation has been observed. The test is terminated.

NOTE Under some circumstances, it can be necessary to confirm this result by further investigations in line with ISO 10993-9.

5.4.4.3 Case 2 (No/Yes)

No change in mass balance, but molecular mass/distribution has changed.

Check the bulk sample and debris for degradation products.

5.4.4.4 Case 3 (Yes/No)

Change in mass balance, but no change in molecular mass/distribution.

Polymer is not degraded, fluid phase contains leachables which shall be assessed according to other relevant parts of ISO 10993.

5.4.4.5 Case 4 (Yes/Yes)

Change in mass balance and change in molecular mass/distribution.

Identify and quantify leachables and polymer degradation products from the fluid phase and check the bulk sample and debris for degradation products, then implement ISO 10993-1 and/or ISO 10993-17 as applicable.

6 Test report

The test report shall include the following information:

a) description of the test material, batch or lot number, dimensions and number of samples tested;

b) test solution and conditions;

c) detailed description and justification of the test methods used, including (where appropriate) specificity, sensitivity, detection and quantification limits;

d) method used to determine mass loss, including precision;

e) mass/volume ratio of sample, shape of sample;

f) sample pre-treatment and drying method;

g) selected pH;

h) test temperature;

i) test periods;

j) test results;

   1) mass balance;

   2) molecular mass/distribution (crosslink density);

   3) results of tests run on the solution, debris and/or bulk polymer;

   4) identified degradation products;

   5) rationale for final decision;

k) conclusions.

Annex A
(informative)

Analytical methods

The following analytical methods are suggested for the characterization of the polymeric material and/or degradation products, if appropriate:

a) solution viscometry (molecular mass average, branching);

b) swellability (crosslink density);

c) rheology (melting range, melt viscosity, thermal stability, molecular mass distribution);

d) chromatographic methods (e.g. gas and/or high-performance liquid chromatography for residual monomers, additives and leachables; size exclusion/gel permeation chromatography for molecular mass averages and changes in molecular mass distribution; mass spectrometry for identification);

e) spectroscopic methods (e.g. ultraviolet spectroscopy, infrared spectroscopy, nuclear magnetic resonance, mass spectroscopy for identity, composition, distributions; atomic absorption spectroscopy for catalyst content, heavy metals);

f) thermal analysis (e.g. differential scanning calorimetry for glass transition, melting range or softening point, blends).

Because a finished medical device can contain several materials from several sources, an intensive literature study and the request for reliable analytical data from the suppliers is strongly recommended to minimize the analytical work. Further guidance can be found in ISO 10993-18 and ISO 10993-19.